REMARKS

In the Office Action dated July 29, 2004, claims 39-46, 51, 56-58, 60-68 and 86-94 are pending and under consideration. Claims 39-46 are rejected under 35 U.S.C. §102(b), as allegedly anticipated by Shamblott et al. (*PNAS* 95: 13726-31, November 1998). Claim 51 is rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Thomson et al. (*Science* 282: 1145-1147, November 1998) in view of Brustle et al. (*Science* 285: 754-756, July 1999). Claims 56-58 and 86 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Thomson et al. in view of Brustle et al. and Stemple et al. (*Cell* 71: 973-985, December 1992). Claims 60-63 and 87 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Thomson et al. in view of Brustle et al, and further in view of Stemple et al. and Ben-Hur et al. (*J. Neurosci.* 18: 5777-5788, August 1998). Claims 64-68 and 88-94 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Thomson et al. in view of Brustle et al. and Ben-Hur et al.

This response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claims 39-46 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Shamblott et al. These claims are drawn to methods of inducing differentiation of stem cells *in vitro* into progenitor cells by obtaining undifferentiated human pluripotent ES cells and providing a controlled differentiating condition which is non-permissive for stem-cell renewal, does not kill cells or induce unidirectional differentiation toward extraembryonic lineages.

According to the Examiner, Shamblott et al. teach the generation of pluripotent human ES cells from cultured human primordial germ cells ("PGCS"). Gonadal ridges from post-fertilization human embryos were collected and the cells were cultured. The cells were

found positive for five immunological markers of ES cells. The immunohistochemical analysis of embryoid bodies collected from a culture of the cells revealed a wide variety of differentiated cell types, including derivatives of all three embryonic germ layers, for example, ectodermal derivatives of cells suggestive of neuroepithelia and antineurofilament cells. Shamblott et al. therefore conclude that the PGCS are pluripotent stem cells that are positive for markers commonly used to identify pluripotent stem cells, that the PGCs have morphology similar to mouse ES and EG cells, maintain a normal and stable karyotype, and can be differentiated into a wide variety of cell types.

In the first instance, Applicants respectfully direct the Examiner's attention to the amendments to claims 39-46. Specifically, claim 39 has been amended to specify the progenitor cells as "neural progenitor cells", which are further defined by the expression of at least one of NCAM, nestin, vimentin or Pax-6, and the lack of expression of Oct-4. Support for this amendment is found at page 42, lines 12-17, and in Figures 3 and 19, for example.

Applicants respectfully submit that the claims, as amended, are directed to methods of inducing somatic differentiation of undifferentiated, pluripotent human embryonic stem cells to neural progenitor cells by providing a controlled differentiating condition. Applicants respectfully submit that Shamblott et al. do not teach controlled differentiating conditions to obtain a homogeneous population of neural progenitor cells. Shamblott et al. teach differentiation of pluripotent stem cells into embryod bodies (EB). EB are understood in the art to contain heterogeneous cell populations comprising derivatives of all three embryonic germ layers. In contrast, the neural progenitor cells, as presently claimed, are a homogeneous population of cells, embodied in the spherical structures described in the specification. A very high percentage of the cells in the population express N-CAM, vimentin, nestin and Pax-6, and

lack expression of Oct-4. Applicants submit that claim 39 presently recites these expression characteristics of the neural progenitor cells.

Applicants further respectfully submit that Shamblott et al. do not teach a controlled differentiating condition as characterized in the present claims. The conditions disclosed by Shamblott et al. apparently resulted in full differentiation. Shamblott et al. do not teach halting the differentiation process at an intermediate stage of cell differentiation, e.g., neural progenitor cell stage, as claimed in the present invention. In this regard, Applicants observe that in Shamblott et al., the embryonic stem cells were grown continuously in a media supplemented with serum. Hence, the resultant embryoid bodies are believed to be different from the neural spheres described in the present application, which resulted from culturing human ES cells in a serum-free media.

In contrast, the neural progenitor cells of the present invention are clearly an intermediate cell type, characterized by the lack of expression of Oct-4. The lack of expression of Oct-4 indicates that the cells have moved on from an undifferentiated state, but are not quite differentiated. Further, Applicants respectfully submit that controlled differentiating conditions are taught in the present specification and are delineated in the instant claims. See claim 46, for example.

To delineate a preferred method of the present invention which involves culturing cells in serum free media, Applicants have added claim 95, which is fully supported by the disclosure on page 40 – 41 of the specification. No new matter is introduced.

In view of the foregoing, Applicants respectfully submit that Shamblott et al. do not teach the claimed methods. Withdrawal of the rejection of claims 39-46 under 35 U.S.C. §102(b) based on Shamblott et al., is therefore respectfully requested.

Claim 51 is rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Thomson et al. in view of Brustle et al.

The Examiner contends that Thomson et al. teach the isolation of human pluripotent embryonic stem (ES) cells. According to the Examiner, Thomson et al. offer a motivation to those skilled in the art by stating that human ES cells can be differentiated into somatic cells to provide a source of cells for drug discovery and transplantation therapies. The Examiner alleges that Thomson et al. state that the progresses made in the differentiation of *mouse* ES cells into somatic cells will be useful to direct the differentiation of *human* ES cells.

With respect to Brustle et al., the Examiner contends that Brustle et al. teach methods of inducing differentiation of mouse ES cells to glial precursors, a somatic progenitor cell, by culturing mouse ES cells in the presence of FGF2 and PDGF-AA in DMEM/F12 media and on polyornithine coated dishes. The withdrawal of growth factors caused the progenitor/stem cells to differentiate into oligodendrocytes.

Therefore, the Examiner contends that it would have been obvious to those skilled in the art, to culture human ES cells, as taught by Thomson et al., in DMEM/F12 media in the presence of FGF2 and PDGF-AA on polyornithine to form glial precursors, and then in the absence of the growth factors to induce the formation of astrocytes and oligodendrocytes, as taught by Brustle et al. The Examiner concludes that Thomson et al. and Brustle et al. provide sufficient suggestion, teaching and motivation to reach the claimed invention.

Applicants respectfully submit that claim 51 has been amended to further define that the progenitor cells are neural progenitor cells. Thus, claim 51, as presently amended, is drawn to a method of inducing differentiation of *neural progenitor* cells to somatic cells by obtaining *neural progenitor* cells derived from undifferentiated *human* pluripotent ES cells, culturing the

progenitor cells on an adhesive substrate in the presence of *serum free media* and growth factors, and inducing differentiation by withdrawal of the growth factors.

Applicants respectfully submit that the references in combination fail to teach or suggest the claimed method in the following aspects. In the first instance, neither Thomson et al. nor Brustle et al. teach or suggest inducing differentiation of progenitors by first growing the progenitor cells in a *serum-free media* in the presence of growth factors, and then withdrawing the growth factors. In Thomson et al., human ES cells were allowed to differentiate in a media containing 20% fetal bovine serum. See Thomson et al. at page 1147 under numeral 6. Similarly, in Brustle et al., the embryoid bodies (i.e., aggregates of differentiated cells of various types) were formed in a media containing 20% fetal bovine serum. See Brustle et al. at page 756 under numeral 7. Therefore, Applicants respectfully submit that neither reference describes the use of a serum-free media or suggests that such media would be beneficial for maintaining neural progenitors before induction of differentiation by withdrawal of the growth factors. Applicants respectfully submit that the combination of growth factors and a serum-free media is used to select, support, enrich and expand neural progenitor cells, which are then induced to differentiate by withdrawal of the growth factors.

Furthermore, Applicants respectfully submit that neither reference teaches or suggests *neural progenitor cells* derived from undifferentiated *human* pluripotent ES cells, much less a method of inducing differentiation of such progenitor cells as presently claimed. Although Thomson et al. may have suggested that human ES cells can be differentiated into somatic cells, Thomson et al. do not provide any substantive teaching of any *in vitro* differentiation conditions. In addition, there is no indication in Thomson et al. that any neural progenitor cells were actually derived *in vitro* from undifferentiated human ES cells. The glial precursors, as disclosed in

Brustle et al., were derived from *mouse* ES cells. Although Thomson et al. suggest that the progresses made in the differentiation of mouse ES cells into somatic cells will be useful to direct the differentiation of human ES cells, neither Thomson et al. nor Brustle et al. teach or suggest *how* the information obtained from differentiating *mouse* ES cells can be used to successfully differentiating *human* ES cells. It is observed that Shamblott et al. allegedly identified neural cell types in Embryoid Bodies (EB's), which were generated from *mouse* ES cells only with the addition of hrLIF to the cell culture. See page 13729, left column, first full paragraph. In contrast, according to the present application, e.g., at pages 64-65, LIF is not required for the induction of somatic differentiation of *human* embryonic stem cells. Given that human ES cells and mouse ES cells have different requirements for growth and differentiation, those skilled in the art would not have had reasonably expected that the conditions for differentiating mouse ES cells, as disclosed in Brustle et al., would be applicable to differentiating human ES cells, e.g., human ES cells disclosed by Thomson et al.

Applicants respectfully submit that for claimed subject matter to be obvious in view of a combination of prior art references, the prior art must suggest the combination to one of ordinary skill in the art and reveal that one of such skill would have a reasonable expectation of success in carrying out the invention. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). In particular, the fact that references can be combined does not make the combination obvious unless the prior art also contains something to suggest the desirability of that combination. In re Imperato, 486 F.2d 585 179 USPQ 730 (C.C.P.A. 1973); In re Sernaker, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983).

In view of the foregoing, Applicants respectfully submit that the rejection of claim 51 under 35 U.S.C. §103(a) based on Thomson et al. and Brustle et al., is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 56-58 and 86 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Thomson et al. in view of Brustle et al. and Stemple et al. These claims are drawn to a method of inducing differentiation of progenitor cells to somatic cells by obtaining neural progenitor cells derived from undifferentiated human pluripotent ES cells, culturing the neural progenitor cells on an adhesive substrate which comprises poly-D-lysine and laminin in the presence of serum free media (claims 56, 58 and 86), and further culturing the neural progenitor cells in the presence of retinoic acid (claims 57, 58 and 86).

Similar to the §103(a) rejection of claim 51 above, the Examiner contends that Thomson et al. teach the isolation of human pluripotent ES cells and suggest that human ES cells can be differentiated into somatic cells. The Examiner also contends that Brustle et al. teach inducing differentiation of mouse ES cells to glial precursors, astrocytes or oligodendrocytes. Further, the Examiner contends that Stemple et al. teach that the differentiation of neural crest multipotent cells into neurons required growth on poly-D-lysine. The Examiner also alleges that Stemple et al. teach growing neural stem cells in the presence of retinoic acid. Moreover, the Examiner states that laminin was known at the time of the instant invention to be an adhesive substrate for neural cell growth and differentiation. The Examiner therefore concludes that it would have been obvious to those skilled in the art to culture human ES cells, as taught by Thomson el al., under conditions taught by Brustle et al. to form neural precursors, and growing the precursors in media comprising retinoic acid and on poly-D-lysine and laminin-coated plates to induce neuronal growth, as taught by Stemple et al.

Applicants have amended claim 56 to add that the neural progenitors are cultured "in the presence of a serum-free media" on an adhesive substrate comprising poly-D-lysine and laminin. Support for this amendment is found in the specification at page 42, lines 28-30 and page 45, lines 8-11, for example.

As submitted above, Applicants respectfully submit that neither Thomson et al. nor Brustle et al. teach or suggest *neural progenitor cells* derived from undifferentiated *human* pluripotent ES cells, much less a method of inducing differentiation of such progenitor cells as presently claimed. This deficiency is not cured by Stemple et al., whose teaching is directed to *rat* neural stem cells.

Further, as submitted above, neither Thomson et al. nor Brustle et al. teach or suggest inducing differentiation of progenitors by growing the progenitor cells in a *serum-free media.* Stemple et al. do not teach culturing progenitor cells in a serum-free media either. There is simply no recognition in any of the cited references of the criticality of a serum-free media to differentiation of neural progenitor cells.

Moreover, Applicants respectfully submit that the teaching in Stemple et al. with respect to differentiation conditions is directed to neural crest cells isolated from rat E10.5 trunk neural tubes. Neural crest cells are *not* neural progenitor cells derived *in vitro* from pluripotent embryonic stem cells presently claimed. Those skilled in the art would not have had any reasonable expectation that the conditions for differentiating rat neural crest cells would be applicable to neural progenitor cells derived *in vitro* from pluripotent embryonic stem cells, let alone to human neural progenitor cells derived *in vitro* from human pluripotent embryonic stem cells, as presently claimed.

Furthermore, with respect to laminin, although laminin was known to be an adhesive substrate for neural growth and differentiation, there is no teaching in the prior art to apply laminin to human neural progenitor cells derived *in vitro* from human ES cells. Those skilled in the art would simply not have had a reasonable expectation that this application would be successful.

Applicants respectfully submit that Thomson et al., Brustle et al. and Stemple et al., taken singularly or in combination, do not teach differentiation of human ES cell-derived neural progenitor cells by culturing in the presence of serum-free media and further culturing in an adhesive substrate, as presently recited. Applicants respectfully submit that the Examiner has used the aid of hindsight in evaluating the present invention to support an incorrect finding of obviousness under 35 U.S.C. §103. The fact that references can be combined does not make the combination obvious unless the prior art also contains something to suggest the desirability of that combination. In re Imperato, 486 F.2d 585 179 USPQ 730 (C.C.P.A. 1973); In re Sernaker, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983). For claimed subject matter to be obvious in view of a combination of prior art references, the prior art must suggest the combination to one of ordinary skill in the art and reveal that one of such skill would have a reasonable expectation of success in carrying out the invention. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). In the present case, even assuming, *pro arguendo*, the cited references had provided sufficient motivation, the requisite reasonable expectation of success is still lacking. At best, the references cited by the Examiner merely provide an invitation to experiment. While it may be "obvious to try" to prepare the present combinations of promoters, "obvious to try" is not the standard under 35 U.S.C. §103. In re Fine, 837 F.2d 1071, 1075, 5 USPQ 2d 1596, 1599 (Fed. Cir. 1988).

In view of the foregoing, Applicant respectfully submit that the rejection of claims 56-58 and 86 under 35 U.S.C. §103(a) based on these three references, is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 60-63 and 87 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Thomson et al. in view of Brustle et al, and further in view of Stemple et al. and Ben-Hur et al. These claims are drawn to methods of inducing differentiation of neural progenitor cells by obtaining neural progenitor cells derived from undifferentiated human pluripotent ES cells, culturing the progenitor cells on an adhesive substrate which comprises poly-D-lysine and fibronectin in serum free media in the presence of PDGF-AA and bFGF (claim 60), or in the presence of PDGF-AA, bFGF, and EGF (claim 61), and culturing the progenitor cells further in the presence of T3 (claim 62), wherein the progenitor cells are induced to differentiate into glial cells (claims 63 and 87).

The Examiner contends that Ben-Hur et al. teach culturing neural progenitor cells in the presence of EGF, which led to the production primarily of astrocytes and oligodendrocytes. Further, the Examiner contends that Ben-Hur et al. also teach culturing neural stem cells in the presence of T3, which led primarily to astrocyte and oligodendrocyte production. The Examiner then concludes that at the time of the present invention, it would have been obvious to the ordinary artisan to culture human ES cells, as taught by Thomson et al., in a media disclosed by Brustle et al. to form glial precursors and to further differentiate the precursors, and grow the precursors on poly-D-lysine and fibronectin coated plates to enhance the presence of neurons in the differentiated cells, and further culture such cells in the presence of EGF and T3 to produce a culture of neuronal cells, oligodendrocytes and glia cells, as taught by Ben-Hur et al.

In response, Applicants respectfully reassert that none of Thomson et al., Brustle et al. or Stemple et al. teach or suggest *neural progenitor cells* derived from undifferentiated *human* pluripotent ES cells. Applicants respectfully submit that this deficiency of Thomson et al., Brustle et al. and Stemple et al. is not cured by Ben-Hur et al. In Ben-Hur et al, the starting materials were cells isolated from neural tissue of newborn rats. Ben-Hur et al. do not teach *human* neural progenitor cells derived from human pluripotent *embryonic stem* cells. Those skilled in the art would not have had any reasonable expectation that the conditions for differentiating cells derived from rat newborns' neural tissue, as disclosed by Ben-Hur et al., would be applicable to neural progenitor cells derived from *human embryonic* stem cells.

Furthermore, similar to Thomson et al., Brustle et al. and Stemple et al., Ben-Hur et al. do not teach or suggest inducing differentiation of progenitors by first growing the progenitor cells in a *serum-free media*, as presently claimed.

In view of the foregoing, Applicants respectfully submit that the rejection of claims 60-63 and 87 under 35 U.S.C. §103(a) based on the combination of Thomson et al., Brustle et al, Stemple et al. and Ben-Hur et al., is improper. Withdrawal of the rejection is therefore respectfully requested.

Claims 64-68 and 88-94 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Thomson et al. in view of Brustle et al. and Ben-Hur et al. These claims are essentially drawn to methods of producing an enriched population of neural progenitor cells from undifferentiated human pluripotent ES cells.

The Examiner's position with respect to these references has been summarized hereinabove. Essentially, the Examiner concludes that at the time of the present invention, it would have been obvious to those skilled in the art, to culture human ES cells, as taught by

Thomson et al., under conditions taught by Brustle et al. and Ben-Hur et al., to produce an enriched population of neural progenitor cells.

In response, Applicants respectfully submit that there is simply no showing or suggestion in any of the cited references to develop a homogeneous population of neural progenitor cells that are of an intermediate cell type, and that are not pluripotent cells but are multipotent neural progenitor cells that will always provide a neural type of cells, either neurons, astrocytes or oligodendrocytes.

Furthermore, Applicants respectfully submit none of the cited references teach or suggest obtaining neural progenitor cells from human pluripotent stem cells using a serum-free media. It is a unique recognition by the present inventors that differentiation of embryonic stem cells to neural progenitor cells is enhanced by culturing the stem cells in a serum-free media.

Additionally, there is no indication in any of the references that the conditions disclosed in Brustle et al. and Ben-Hur et al., which relate to mouse and rat stem cells, would be applicable to successfully develop neural progenitor cells from *human embryonic* stem cells. Those skilled in the art simply would not have had a reasonable expectation of success in combining the teachings of the cited references.

Therefore, Applicants respectfully submit that the rejection of claims 64-68 and 88-94 under 35 U.S.C. §103(a) based on the combination of Thomson et al., Brustle et al. and Ben-Hur et al., is improper. Withdrawal of the rejection is therefore respectfully requested.

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

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